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A CRITICAL STUDY OF THE METHODS IN CURRENT USE FOR THE DETERMINATION OF FREE AND ALBUMINOID AMMONIA IN SEWAGE.*

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INTRODUCTION.

THE sanitary analysis of water has engaged the attention of chemists for many years, and has been developed to a high degree of perfection. It aims to show the present condition and past history of the water in question, and, in spite of the fact that certain of its most important determinations are of comparative value only, its results, in the hands of a competent chemist, are of no uncertain value. Unfortunately perhaps for the cause of sewage disposal, the methods so long in use for water analysis have been adopted, almost without change, for the examination of sewage and of the products of sewage-disposal works. The analysis of a sewage or effluent of a sewage filter is not undertaken for the purpose of determining the past history of the sample or its present fitness as a drinking water. The problems to be solved are engineering problems. It is gratifying, therefore, to note that the engineer is appreciating more and more the fact that the data upon which he must depend in designing a purification plant are largely chemical.

The question is often asked: "Can you not give us more information on this matter? We wish definite figures which can be used in the compilation of formulæ so that the design of a sewage-disposal plant will be as definite a problem as the construction of a bridge." In fact, the engineering demands have been so exacting and the chemical data so unreliable that discredit has been thrown upon much of the chemical work.

We must frankly confess that we cannot, with our present knowledge at least, give any such absolute information; and it is almost a matter of certainty that the engineer can never solve his problem in this manner.* The laws of stress and breaking strength are fixed, and capable of experimental determination and of exact application. The sewage problem is never twice the same. Analyses of the sewages of two communities, apparently the same in many respects, show great differences. Climatic conditions exert the greatest influence. The presence of manufacturing wastes in the sewage complicates the problem, and even the quality of the water supply and the geological formation of the region may alter the conditions profoundly. It is on account of these variations that the problem cannot be handled by rigid mathematics. The variables are so many that probably no amount of experimentation could ever supply the necessary constants for use in an engineer's formula.

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*Read at the meeting of the Laboratory Section of the American Public Health Association, October 26, 1903.

But while it is true that we cannot give all that is desired in the way of analytical data, there seems to be a growing conviction in the minds of many that our present methods of chemical analysis are inadequate, if not actually misleading at times, and that the time has come for a revision of the methods now employed, and possibly a recasting of the whole scheme of sewage analysis, taking it out of the old familiar ruts, into which it seems to have fallen, and raising it more nearly to the plane of modern engineering requirements.

What, then, should the chemical analysis of sewage include, in view of its functions as indicated above? The analysis should, first, show the total amount of organic matter to be dealt with in the course of twenty-four hours of an average day, and, if possible, the amount of variation from this average day's result to be expected during the year. Next, the nature of the organic matter must be known; that is, whether it is nitrogenous, or carbonaceous, etc. The amount of oxygen available in the sewage, either as dissolved gaseous oxygen, or as nitrate or nitrite, is another important element in the analysis. If the sewage is strongly acid or alkaline, the fact is of importance; but in the ordinary slightly alkaline domestic sewage the determination of alkalinity is of little moment. The determinations of chlorine, iron, phosphorus, and other mineral ingredients give no information not otherwise obtainable, except in connection with precipitation works, when any abnormal amounts of a mineral substance may be of a considerable importance. A knowledge of the total mineral matter may be of value in certain processes.

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*Mr. W. D. Scott-Moncrieff[1] has recently proposed a standard apparatus for use in determining certain constants in sewage. The use of such constants, however, does not seem to have met with favor in England.

The analysis of sewage is made, in short, to give the engineer information to assist him in the design of a purification plant. By comparison of its figures with those from plants already in operation, it is possible to obtain some information as to the probable results to be expected by treating the sewage in question. Such comparisons are often misleading, however, and cases might be cited in which two sewages, agreeing very closely in chemical analysis, yield widely differing results after similar treatment.

The information above outlined as desirable in a sewage analysis is obtained, in water analysis, by the determination of the free and albuminoid ammonias, nitrates, nitrites, oxygen consumed (or absorbed), oxygen dissolved, alkalinity, and fixed solids. It has been assumed that the same methods applied to sewage will give the same kind of information. That this is not necessarily the case I hope to show in this paper, and that I am not alone in distrusting the analytical results as now obtained will be shown by a quotation from a recent paper by Mr. G. W. Fuller.[2] Mr. Fuller says:

> It seems to the writer that the time is ripe for a thorough review and comparison of methods for determining organic matter in sewage on a scale as comprehensive as that made in connection with natural waters under Professor Mallet for the National Board of Health over twenty years ago. To the thought of the writer, the future of sewage chemistry cannot depend upon the question of convenience of manipulation in the laboratory, or that of the degreee of skill of the laboratory assistants.

And again:

> As to the part played by analytical data in the development of matters of sewage disposal, their value has unquestionably been great. In the opinion of the writer, however, methods employed for studying the composition of sewage in its various phases have fallen somewhat into a rut. It seems questionable whether the amount of added knowledge which may be obtained from a continuance of present methods year after year is going to be commensurate with the cost. In fact, it seems that the time is approaching

when it is worth while to consider a recasting of the program for sewage analysis. This may be thought to be unwise, because future data would not be comparable with the past. This objection is readily met by stating that there should be omitted in the future no determination of value. In fact, it is more complete data which are now needed.

The work here presented is the first portion of a study which will ultimately cover the whole field of sewage analysis. It was carried out at the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology. The sewage used is a representative domestic sewage, coming from the large trunk sewer of the Boston Main Drainage. In planning and carrying out the work, the attempt has been made to show by decisive experiments just what a given determination shows us, to what errors it is liable, and what modifications, if any, are necessary in order that the determination shall yield the best results both from the chemical and from the engineering points of view.

THE FREE AMMONIA.

In the determination of the free ammonia as ordinarily carried out a measured quantity of the sewage is diluted with water, made alkaline with sodium carbonate, and subjected to indirect distillation with live steam. The ammonia is determined in the distillate by the Nessler process. In this procedure we have to consider errors in the distillation and those in the reading. The former might occur in two ways: (1) the whole of the free ammonia present might not be recovered in the distillate; and (2) substances might be decomposed during the distillation which would give off an additional amount of ammonia.

A number of determinations of the ammonia in solutions of ammonium chlorid of known strength have shown that the whole of the free and saline ammonia is recovered, the small errors observed being about equally distributed above and below the true value, and being easily explained by other errors in the process.

On the other hand, it has been found during the present study that a large number of proteid substances are partially broken down during the distillation with sodium carbonate, giving too high a value to the ammonia readings. Among the pure substances tested in this way the following gave positive results as shown, expressed as percentages of the total nitrogen given off as free ammonia:

Substance	Percentage of Total Nitrogen Found as Free Ammonia
Urea	2.0
Gelatin	1.4
Casein	0.68
Egg albumen	3.2
Peptone (Witte's)	1.4
Naphthylamine	0.1

That this error is of great significance in the analysis of sewage is shown by an extended study on the sewage itself, and on the effluents of sewage purification processes. About fifty samples were analyzed in the regular way, distilling off the free ammonia with sodium carbonate, and then adding alkaline permanganate of potassium and distilling the albuminoid ammonia. A second sample was, in each case, filtered through paper and treated in a similar manner. A third sample was treated with an excess of sodium hydrate and a few drops of a 10 per cent. solution of copper sulphate, and allowed to settle, the clear supernatant liquor being then diluted and nesslerized directly for free ammonia. With very few exceptions the values obtained were lowest in the case of the direct reading, and highest with the unfiltered sample, the filtered sample generally lying between the other two. Moreover, the excess of ammonia found in the distilled sample over that from the direct reading was in all cases roughly proportional to the amount of albuminoid ammonia in the former. The average of fifty such analyses is: raw, 18.8 parts per million; filtered, 17.5 parts; direct, 16.6 parts; albuminoid ammonia—raw, 2.8 parts; filtered, 1.9 parts. The average excess of the free ammonia in the raw samples over that by the direct reading is thus 13.2 per cent. of the direct reading.

Experiments will be presented later in this paper showing that the results obtained by this direct process are much nearer the true values than any others. The subject is brought up at this time merely to call attention to a serious error in the determination as practiced at present.

Passing now to a consideration of the errors in reading, we find here many opportunities for inaccuracies.

Temperature.—Attention has been called by Hazen and Clark[3] to the fact that the ammonia standards and the distillates must be at the same temperature to secure correct readings. As long as the regular standards, made up each day, are used, the usual practice of allowing the distillate to stand over night was sufficient to secure this condition. But with the advent of permanent standards the question of temperature becomes one of importance. It has been found that the standards themselves remain practically constant in color during any changes in temperature that may occur in the laboratory. Therefore, if accuracy is to be had in reading ammonias against these standards, the reading must be done at the temperature at which the standards were originally made to compare with the ammonia standard solution, or a temperature correction must be applied.

Dividing the tubes for reading.—When 10 c.c. are taken for an analysis, an amount necessary in order to get satisfactory reading of the albuminoid ammonia, the amount of free ammonia coming off in the first tube is always too high for direct nesslerization. It is customary to allow the tubes to stand over night, and the next morning to take out a suitable quantity, generally 10 c.c., and dilute to 50 c.c. for nesslerization. Samples taken from the top and bottom of several such tubes gave identical readings, showing that diffusion was ample in the time allowed, about fifteen hours. If the tubes are read directly after distillation, however, there is a possibility of error at this point, which must be guarded against by a careful mixing of the contents of the tube.

Variation in the diameters of the nessler tubes.—Considerable variation is met with in the diameters, and consequently in the lengths, up to the mark, of the nessler tubes, as they are found in the market. Our laboratory stock, purchased at two different times from the same dealer, was looked over, and tubes having the maximum and minimum lengths were selected for a comparison. Readings of the same solution with these two tubes gave values of 3.4 and 3.9 c.c. of ammonia standard, respectively—a variation of over 10 per cent. The tubes were extreme cases, it is true, but the stock included tubes of all lengths between. The only way, obviously, to correct this error is to throw out all tubes which in length do not agree sufficiently well among themselves to give results of the desired accuracy. For very accurate work the plan suggested by Fowler is to be recommended; that is, to select a set of tubes for the standards which shall be all of exactly the same length, and have one extra tube of this length for the readings. All distillates are poured into this tube before reading. This plan is somewhat cumbersome for routine laboratory work, but is the only one which will give strictly accurate results, unless a sufficiently large number of tubes can be obtained of the same diameter.

Color in the glass.—The writer has seen tubes of such a poor quality of glass that a color amounting to 0.3 c.c. of ammonia standard was imparted to the distillate. Such tubes should never be used, since the variation in the thickness of the glass will give a corresponding variation in the color, and the readings will be in error by this amount.

A METHOD FOR THE DIRECT READING OF THE FREE AMMONIA.

Considerations previously given concerning the formation of free ammonia during the distillation indicate the desirability of some method for determining this important constituent in a more direct and more accurate manner.

Farnsteiner,[4] and his associates at the Chemical and Hygienic Institute, have proposed such a method, as follows: The sewage is treated with sodium hydrate solution and allowed to settle. A portion of the supernatant liquor is removed and nesslerized directly. In the presence of hydrogen sulphide a few drops of a solution of zinc acetate is added.

After a trial of different combinations of precipitants, I have decided upon the following method as best suited to all-around work at a sewage experiment station.* The solutions required are

1. A 10 per cent. solution of copper sulphate.
2. A 10 per cent. solution of lead acetate.
3. A saturated solution of potassium hydrate.

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*This successful working out of this method is largely due to the careful work of Mr. G. R. Spalding, research assistant at the Sanitary Research Laboratory.

Fifty c.c. of the sewage to be tested are mixed with an equal volume of water, placed in a short nessler tube about six inches long, and a few drops of the copper solution added. After a thorough mixing, 1 c.c. of the potassium hydrate solution is added and again thoroughly mixed. The tube is then allowed to stand for a few moments, when a heavy precipitate falls to the bottom, leaving a colorless supernatant. An appropriate amount of this solution, usually 2–5 c.c., is then taken, made up to 50 with best water, and nesslerized. We have varied the procedure of Farnsteiner in substituting lead acetate for the zinc salt which he used, and in precipitating a diluted sewage instead of the straight sample. The lead acetate, we find, is a better precipitant, especially for our septic tank effluents, where there is a large amount of hydrogen sulphide present, in which case a few drops of the lead acetate solution is added with the copper solution. The sewage is diluted before precipitation to prevent loss by absorption, and because we find that some effluents are so high in ammonia that there is a noticeable escape of the gas on adding the caustic. Our strongest effluents, however, showed no escape of ammonia, on testing with hydrochloric acid, when properly diluted as described. As to precipitants, no general method can be laid down at present for all cases, but, with a little practice, it is comparatively easy to get the right combination of the three solutions mentioned to bring about the best results.

It has been stated previously that this method yields results nearest to the true ammonia value, The evidence for this statement is as follows: (1) The results were invariably lower than those from the distillation processes. Moreover, when raw sewage and sewage filtered through paper were distilled, the filtered sample in nearly every case gave a result lying between that of the direct reading and that of the raw sample distilled. An average of fifty such analyses gave, for the direct reading, 16.6 parts per million; for the filtered sample, 17.5 parts; and for the raw sample, 18.8 parts. It appears from this that the increase in the values obtained for the distilled samples is due to a decomposition of the nitrogenous matter of those samples, and the increase is about proportional to the amount of albuminoid ammonia given in the two cases. (2) Experiments with pure nitrogenous substances, likely to be fond in sewage, show that they do give off free ammonia on distillation with sodium carbonate. I have given a list of the substances used and the results obtained. As an amplification of this experiment an artificial sewage was made up of peptone, urea, albumin, vitilline, casein, and salt. The ammonia was determined by direct reading, and found to be nil. Enough ammonium chloride solution was now added to bring up the free ammonia to 10 parts per million, and a sample was distilled, while a second sample was precipitated and read directly. The direct reading was 10 parts, while the distillation gave a value of 10.7 parts. That the difference is not so great as is observed in sewage is due to the fact that in the latter we have exceedingly unstable compounds, bodies which are already in the process of conversion to free ammonia, and these are more readily broken down than the more stable bodies with which I have experimented. (3) This conclusion is in harmony with the evidence of Frankland,[5] Thatcher,[6] Schittenhelm,[7] and others, to the effect that certain bodies, particularly amines and urea, will give off ammonia on distillation with sodium carbonate.

Dr. Frankland, in a paper on the analysis of water, quotes experiments on the analysis of polluted waters, in which he shows that even in that case the free-ammonia determination is seriously in error, owing to the decomposition of certain substances on distillation, notably urea. Thatcher, in a thesis at the Massachusetts Institute of Technology, has also shown that amines and other nitrogenous bodies break down on distillation in alkaline solution, forming free ammonia. Schittenhelm obtained similar results in the distillation of feces. Cohn[8] states in his work on *Reagents* that aldehydes give a brownish coloration with Nessler's reagent.

THE ALBUMINOID AMMONIA.

After the distillation of the free ammonia, as previously described, 20 c.c. of an alkaline solution of potassium permanganate is added to the sample, and the distillation continued, taking off two more tubes, or 100 c.c. The ammonia thus recovered in the distillate is read in the same way as the free ammonia and designated "albuminoid ammonia." It is, in fact, a portion of the nitrogen of the nitrogenous matter of the sewage which is given off in the distillation as ammonia.

The albuminoid-ammonia test was first proposed by Wanklyn in 1867. It was first announced that it gave the whole of the organic nitrogen. Then in a later paper Wanklyn claimed that he obtained a constant proportion of that total, about two-thirds of it. In the following year E. Frankland[5] showed by a series of experiments with the process that the ratio was not two-thirds, and, in fact, that it was not a constant figure at all. However, owing to its simplicity and roughly relative results, the test has been retained in the routine analysis of potable waters, and thence has been adopted without change in the analysis of sewage.

In water analysis it is claimed, and in the case of unpolluted waters it is doubtless true,[9] that the amount of nitrogen obtained in the distillation is a constant proportion of the total, and hence the figure is of value in the interpretation of the analysis. This relation does not, however, hold even approximately with sewages and sewage effluents. Besides the nature of the substance itself, there are certain details in the process which influence the result. Experiments are here reported showing the effect of varying some of these factors.

Concentration of the permanganate in the solution.—The amount of permanganate solution added is found to have an influence upon the result. A sample of casein in solution, yielding by the regular method 8.8 parts of albuminoid ammonia per million, gave with double the amount of permanganate a value of 10.4 parts, and with one-half the correct amount a value of 4.0 parts; or, expressed in percentages, the higher concentration gave 118 per cent., and the lower 45 per cent., of the true value obtained by the standard procedure. It will be apparent that if the amount of sewage solution added varies, there will be a similar variation in the concentration of the permanganate with its consequent error. A sample of sewage treated in the same way gave similar results.

Rate of distillation.—Stress is usually laid upon this point in the text-books. A little consideration will show, however, that the temperature will vary little, if at all, with a variation in the rate of distillation, and that, since the reaction goes on rapidly at first, and continually decreases in rate, a slower distillation is equivalent to an addition of time at the end of the distillation, when the reaction is practically completed. In short, it would appear that practically the same amount of ammonia will be yielded in a given time, whatever may be the rate. Within ordinary limits, this is found to be the case. The solution of casein used in the last experiment was also used in this one. A distillation was made at the ordinary rate of three tubes, or 150 c.c., in twenty-five minutes, and a second one at a rate of three tubes in fifty minutes. The second distillation gave a reading of 106 per cent. of the first, and the amount coming off in the first twenty-five minutes of the second case exactly equaled that obtained in the total twenty-five minutes in the first. Experiments with sewage at three different rates confirmed these results. It is therefore the *time* of distillation rather than the rate which has an influence upon the result.

Solubility of the nitrogenous substance.—It might be expected that the solubility of the substance giving up the ammonia might exert some influence upon the rate of the reaction. To test this point egg albumen was used. A solution was made of this substance, and a portion taken for analysis. A second sample was heated gently with mercuric chloride to coagulate it, and then submitted to analysis. The results obtained were 5.40 and 5.24 parts of nitrogen per million respectively. This experiment has some bearing upon the results in filtration work, and especially in the septic tank effluents; for, other things being equal, the soluble substance will give off relatively more of its nitrogen than the insoluble.

Percentage of total nitrogen recovered as albuminoid ammonia.—Having shown some of the minor variations which may be expected in a sewage analysis, I will now report some work undertaken to find out just what percentage of the total nitrogen we may expect to get off in the albuminoid-ammonia process, under the standard conditions of analysis. I will assume that the Kjeldahl process for total organic nitrogen gives that figure with sufficient accuracy. Some doubt has recently been thrown upon the correctness of this view[10, 11] but for small quantities of nitrogen I believe the Kjeldahl results to be accurate. In some control experiments made with the Gunning modification of the Kjeldahl process and, in parallel, with a much simpler modification, to be described later, theoretical results were obtained within the accuracy of the Nessler reading, urea and asparagin being used. Next a number of substances likely to be found in sewage were analyzed by the albuminoid-ammonia process, and by the Kjeldahl, and the percentages of the total nitrogen given off in the former were calculated. The results were as follows;

Substance	Percentage of Total Nitrogen Recovered as "Albuminoid Nitrogen"
Egg albumen	32
Vitilline	18
Casein	21
Gelatin	42
Grass extracts —	
Cold water	54
Hot water	48
Chloroform (chlorophyll)	29
Peptone (Witte's)	30
Asparagin	73
Urea	4
Tap water	45

Similar work on sewages gave, as an average of fifteen analyses, a value of 34.8 per cent., with maximum and minimum values of 15.2 and 54.8 per cent. These wide variations merely show the impossibility of forming any estimate of the amount of nitrogenous material present in a sewage from its albuminoid ammonia. Taking twice the albuminoid as a total, we get a maximum error of 70 per cent.; with three for the factor, the maximum error is 34 per cent.; while, with the formula proposed by Mr. Fuller, a much closer agreement is found in all cases, although the maximum difference in this case is 25 per cent. of the total. The formula includes the free ammonia as well as the albuminoid. It is

$$\text{Total nitrogen} = \frac{12x\ (\text{albuminoid nitrogen})^2}{\text{nitrogen of free ammonia}}.$$

It seems that, in view of these results, and of those quoted by Fuller, the albuminoid-ammonia process, as applied to sewage, has very little value, and might safely be replaced by the Kjeldahl process, or some modification of that process.

The writer finds, by a comparative study, that the simple method used by Palmer[12] in his study of the streams polluted by Chicago's sewage, gives as good results as either the Kjeldahl or the Gunning process. Palmer's method consists merely in digesting with sulphuric acid until the liquid clears up, and then neutralizing and distilling off the ammonia formed. With sewage the method can be made very rapid, half an hour sufcing for the complete digestion. In about half the cases tried in this way it was found, by making up the digested sample to 250 c.c., and taking out 2 c.c., neutralizing and making up to 50 c.c., that the tube could be directly nesslerized. In other cases this could not be done, a turbidity occurring on nesslerizing. Lack of time has prevented a detailed study of this point, but it is believed that some way will be found of overcoming the difficulty, and that it will be possible to nesslerize the solutions directly. If this is so, it will be possible to make a determination of the free ammonia by the direct method, and of the total nitrogen by the Kjeldahl method, in about the same time that is now required for the distillations.

CONCLUSIONS.

In conclusion, I would sum up the more important points shown by this work:

All free and saline ammonia is recovered in the distillate by the ordinary process.

Many albuminoid and other nitrogenous substances decompose on boiling with sodium carbonate, giving off free ammonia, causing an error in both the free and the albuminoid ammonia values.

The percentages of the total nitrogen given off as albuminoid ammonia are so variable that no indication of the total nitrogen can be obtained from the albuminoid-ammonia figures.

A simplified Kjeldahl process and a method for the direct reading of ammonia are suggested which, together, can be made in about the same time as the two distillations as carried out at present, and both of which are constant and accurate.

If, upon further study, the facts brought out here are found to be correct, the question arises whether the time has not come for some such official body as the Standard Methods Committee of this section to make formal inquiry, and, if they deem it wise, to make official recommendations in regard to the best and most desirable method of procedure in sewage analysis.

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